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(57) Abstract

A human monoclonal antibody specific for the melanoma-associated antigen AgGM4 was isolated, sequenced, and characterized. The monoclonal antibody is useful for *in vitro* and *in vivo* diagnosis of melanoma, as well as for therapeutic treatment of the malignancy.

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HUMAN MONOCLONAL ANTIBODY SPECIFIC FOR MELANOMA-ASSOCIATED ANTIGEN AND METHODS OF USE

Field of the Invention

The invention relates generally to monoclonal antibodies and specifically to a human monoclonal antibody specific for an antigen associated with a malignant disease.

Background of the Invention

The basic immunoglobulin (Ig) structural unit in vertebrate systems is composed of two identical "light" polypeptide chains (approximately 23 kDa), and two identical "heavy" chains (approximately 53 to 70 kDa). The four chains are joined by disulfide bonds in a "Y" configuration, and the "tail" portions of the two heavy chains are bound by covalent disulfide linkages when the immunoglobulins are generated either by B cell hybridomas or other cell types.

A schematic of the general antibody structure is shown in Figure 1. The light and heavy chains are each composed of a variable region at the N-terminal end, and a constant region at the C-terminal end. In the light chain, the variable region (termed "V_LJ_L") is composed of a variable (V_L) region connected through the joining (J_L) region to the constant region (C_L). In the heavy chain, the variable region ($V_HD_HJ_H$) is composed of a variable (V_H) region linked through a combination of the diversity (D_H) region and the joining (J_H) region to the constant region (C_H). The V_LJ_L and $V_HD_HJ_H$ regions of the light and heavy chains, respectively, are associated at the tips of the Y to form the antibody's antigen binding portion and determine antigen binding specificity.

The (C_H) region defines the antibody's isotype, *i.e.*, its class or subclass. Antibodies of different isotypes differ significantly in their effector functions, such as the ability to activate complement, bind to specific receptors (e.g., Fc receptors)

present on a wide variety of cell types. cross mucosal and placental barriers, and form polymers of the basic four-chain IgG molecule.

Antibodies are categorized into "classes" according to the C_H type utilized in the immunoglobulin molecule (IgM, IgG, IgD, IgE, or IgA). There are at least five types of C_H genes ($C\mu$, $C\gamma$, $C\delta$, $C\epsilon$, and $C\alpha$), and some species (including humans) have multiple C_H subtypes (e.g., $C\gamma_1$, $C\gamma_2$, $C\gamma_3$, and $C\gamma_4$ in humans). There are a total of nine C_H genes in the haploid genome of humans, eight in mouse and rat, and several fewer in many other species. In contrast, there are normally only two types of light chain constant regions (C_L), kappa (κ) and lambda (λ), and only one of these constant regions is present in a single light chain protein (i.e., there is only one possible light chain constant region for every V_LJ_L produced). Each heavy chain class can be associated with either of the light chain classes (e.g., a $C_H\gamma$ region can be present in the same antibody as either a κ or λ light chain), although the constant regions of the heavy and light chains within a particular class do not vary with antigen specificity (e.g., an IgG antibody always has a $C\gamma$ heavy chain constant region regardless of the antibody's antigen specificity).

Each of the V, D, J, and C regions of the heavy and light chains are encoded by distinct genomic sequences. Antibody diversity is generated by recombination between the different V_H, D_H, and J_H gene segments in the heavy chain, and V_L and J_L gene segments in the light chain. The recombination of the different V_H, D_H, and J_H genes is accomplished by DNA recombination during B cell differentiation. Briefly, the heavy chain sequence recombines first to generate a D_HJ_H complex, and then a second recombinatorial event produces a $V_HD_HJ_H$ complex. A functional heavy chain is produced upon transcription followed by splicing of the RNA transcript. Production of a functional heavy chain triggers recombination in the light chain sequences to produce a rearranged V_LJ_L region which in turn forms a functional $V_LJ_LC_L$ region, i.e., the functional light chain.

The value and potential of antibodies as diagnostic and therapeutic reagents has been long-recognized in the art. Unfortunately, the field has been

hampered by the slow, tedious processes required to produce large quantities of an antibody of a desired specificity. The classical cell fusion techniques allowed for efficient production of monoclonal antibodies by fusing the B cell producing the antibody with an immortalized cell line. The resulting cell line is called a hybridoma cell line.

Recent progress in the field of human monoclonal antibody technology has made it possible to generate numerous hybridomas generating human antibodies of various specificities. Combined with knowledge gained in the understanding of the human immune response to cancer antigens (Lloyd et al. (1989) Cancer Res. 49:3445-3451), several human monoclonal antibodies (MAbs) against tumor associated antigens have been produced and characterized. The reported tumor associated antigens recognized by human MAbs include cell surface (Yoshikawa et al. (1989) Jpn. J. Cancer Res. (Gann) 80:546-553; Yamaguchi et al., Proc. Natl. Acad. Sci. 84:2416-2420; Haspel et al. (1985) Cancer Res. 45:3951-3961; Cote et al. (1986) Proc. Natl. Acad. Sci. 83:2959-2963; Glassy (1987) Cancer Res. 47:5181-5188; Borup-Christensen et al. (1987) Cancer Detect. Prevent. Suppl. 1:207-215), cytoplasmic (Haspel et al. (1985) Cancer Res. 45:3951-3961; Cote et al. (1986) supra; Glassy (1987) supra; Borup-Christensen (1987) supra; Kan-Mitchell et al. (1989) Cancer Res. 49:4536-4541; Yoshikawa et al. (1986) Jpn. J. Cancer Res. 77:1122-1133), and nuclear antigens (McKnight et al. (1990) Hum. Antibod. Hybridomas 1:125-129).

At present, methods of limited effectiveness exist for the diagnosis and/or treatment of various malignancies. Those drugs which are administered generally have severe side effects associated with their use. Accordingly, there exists a significant need to produce monoclonal antibodies which bind to epitopes on malignancy-related antigens. In addition to being potentially useful therapeutic reagents, such antibodies are suitable agents for the diagnosis of malignancies expressing a specific antigen.

Summary of the Invention

The present invention features a human monoclonal antibody specific for a malignancy-related antigen. Specifically, the monoclonal antibody of the invention binds to an antigen, referred to as "AgGM4" herein, found on the surface of malignant melanoma cells. The monoclonal antibody, "GM4-IgG4.λ",also referred to as "GM4" herein, was isolated from a hybridoma generated from pooled lymphocytes obtained from cancer patients and the human fusion partner SHFP-1.

GM4-IgG4.λ comprises a light chain encoded by the DNA sequence of SEQ ID NO:1. The deduced amino acid sequence of the GM4-IgG4.λ light chain (SEQ ID NO:2) is shown with its encoding DNA sequence in Fig. 3. The GM4-IgG4.λ heavy chain is encoded by the DNA sequence of SEQ ID NO:3. The DNA sequence and deduced amino acid sequence (SEQ ID NO:4) are shown in Fig. 4.

The invention further includes an anti-idiotypic antibody of GM4-IgG4.λ.

In a related aspect, the invention features a hybridoma cell which secretes a human monoclonal antibody specific for AgGM4, and having a light chain encoded by the DNA sequence of SEQ ID NO:1, and a heavy chain encoded by the DNA sequence of SEQ ID NO:3.

In one aspect, the invention features a method of detecting the presence of melanoma using a monoclonal antibody. Specifically, a diagnostically effective amount of the detectably labeled antibody of the invention, or a fragment of the antibody thereof, is contacted with a sample suspected of containing melanoma cells, and binding of the antibody determined. Increased binding of the antibody relative to a control sample which does not contain melanoma cells is indicative of melanoma. In one aspect, the detection is conducted *in vitro*, and the detectable label is a radioisotope, a fluorescent compound, a colloidal metal, a chemiluminescent compound, a bioluminescent compound, or an enzyme, for example. In another aspect, the detection is conducted *in vivo*, and the detectable label is a radioisotope or a paramagnetic label, for example.

The invention also provides a method of treating melanoma in an animal by administering a therapeutically effective amount of a monoclonal antibody or fragment of a monoclonal antibody specific for AgGM4. In one aspect, the administration is parenteral. In more specific aspects, the parenteral administration of monoclonal antibody is by subcutaneous, intramuscular, intraperitoneal, intracavity, transdermal, or intravenous injection. The monoclonal antibody may be administered alone, in combination with effector cells, and/or therapeutically labeled. Useful therapeutic labels include but are not limited to radioisotopes, drugs, immmunomodulators, biological response modifiers, lectins, and/or toxins.

In a related aspect, the invention features pharmaceutical composition including a therapeutically effective amount of the monoclonal antibody of the invention with a pharmacological carrier.

One object of the invention is to provide a method of diagnosing melanoma with the use of an antibody specific for the melanoma-related antigen AgGM4.

These and other objects, advantages and features of the present invention will become apparent to those persons skilled in the art upon reading the details of the compositions, composition components, methods and method steps of the invention as set forth below.

Brief Description of the Drawing

Fig. 1 is a schematic showing the basic immunoglobulin structure.

Figs. 2A-2C. Flow cytometry analysis of membrane (S) and cytoplasmic (C) GM4 expression on MENMEL94. Fig. 2A: membrane and cytoplasmic staining on Day 1; Fig. 2B: membrane and cytoplasmic staining on Day 3; Fig. 2C: membrane and cytoplasmic staining on Day 5. The shaded curve represents background fluorescence.

Fig. 3 is the cDNA (SEQ ID NO:1) and deduced amino acid (SEQ ID NO:2) sequence of the GM4 antibody light chain.

Fig. 4 is the cDNA (SEQ ID NO:3) and amino acid (SEQ ID NO:4) sequences of the GM4 antibody heavy chain.

Description of the Preferred Embodiments

Before the present human monoclonal antibody and methods of diagnosis and treatment are described, it is understood that this invention is not limited to the particular methods and antibodies, as such methods and antibodies may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. The publications discussed above are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

Definitions

The term "antibody" as used in this invention is meant to include intact molecules as well as fragments thereof, such as for example, Fab and F(ab')2, which are capable of binding the epitopic determinant. The term "human antibody" means an antibody in which portions of the immunoglobulin molecule are encoded by a DNA sequence derived from a human Ig-encoding nucleic acid sequence. Such human antibodies are desirable for use in antibody therapies, as such

antibodies would elicit little or no immune response in a human patient. Preferably, the human antibody is entirely human, however, "humanized" antibodies are also envisioned.

Antibody fragments retain some ability to selectively bind with its antigen or receptor and are defined as follows:

(1) Fab, the fragment which contains a monovalent antigen-binding fragment of an antibody molecule can be produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain;

(2) Fab', the fragment of an antibody molecule can be obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain; two Fab' fragments are obtained per antibody molecule;

(3) (Fab')2, the fragment of the antibody that can be obtained by treating whole antibody with the enzyme pepsin without subsequent reduction; F(ab')2 is a dimer of two Fab' fragments held together by two disulfide bonds;

(4) Fv, defined as a genetically engineered fragment containing the variable region of the light chain and the variable region of the heavy chain expressed as two chains; and

(5) Single chain antibody ("SCA"), defined as a genetically engineered molecule containing the variable region of the light chain, the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule.

Methods of making these fragments are known in the art. (See for example, Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York (1988), incorporated herein by reference).

The invention provides DNA sequences encoding the monoclonal antibody specific to a melanoma antigen, AgGM4. Also included are DNA sequences which encode AgGM4. The term "DNA sequence" include DNA, cDNA and RNA sequences which encode the monoclonal antibody of the invention. It is

understood that all polynucleotides encoding all or a portion of the antibody or antigen of the invention are also included herein, as long as they encode a polypeptide with the same specificity for AgGM4 or a polypeptide which binds GM4, respectively. Such polynucleotides include naturally occurring, synthetic, and intentionally manipulated polynucleotides. For example, the polynucleotide encoding GM4-IgG4.λ may be subjected to site-directed mutagenesis. The polynucleotides of the invention include sequences that are degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included in the invention as long as the amino acid sequence of GM4-IgG4.λ or AgGM4 polypeptide encoded by the nucleotide sequence is functionally unchanged.

Minor modifications of the recombinant GM4-IgG4.λ primary amino acid sequence may result in antibodies which have substantially equivalent specificity as compared to the GM4-IgG4.λ antibody described herein. Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous. The invention includes such proteins or peptides produced by these modifications, as long as the immunological specificity still exists. Further, deletion of one or more amino acids can also result in a modification of the structure of the resultant molecule without significantly altering its immunological specificity. This can lead to the development of a smaller active molecule which would have broader utility.

By the term "immunological specificity" is meant the ability of an antibody to recognize and bind a specific antigenic epitope. A highly specific antibody binds its antigenic epitope with a high affinity, e.g., an enhanced binding affinity.

The specificity of an antibody can be evaluated, without undue experimentation, to determine if it has the same specificity as the monoclonal antibody of the invention by determining whether the antibody being tested prevents the monoclonal antibody of the invention from binding 1) to the AgGM4 epitope to which GM4 binds, or 2) to a malignant cell expressing the AgGM4

antigen with which the monoclonal antibody of the invention is reactive. If the antibody being tested competes with the monoclonal antibody of the invention, as shown by a decrease in binding by the monoclonal antibody of the invention, then it is likely that the two antibodies bind to the same, or a closely related, epitope.

Still another way to determine whether an antibody has the same specificity as the monoclonal antibody of the invention is to pre-incubate the monoclonal antibody of the invention with the AgGM4 antigen with which it is normally reactive, and determine if the antibody being tested is inhibited in its ability to bind the antigen. If the antibody being tested is inhibited, then in all likelihood it has the same, or a closely related, epitopic specificity as the monoclonal antibody of the invention.

The nucleotide sequence encoding the monoclonal antibody of the invention includes the disclosed sequence and deduced amino acid sequence and conservative variations thereof. The term "conservative variation" as used herein denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acids, or glutamine for asparagine, and the like. The term "conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that the resulting antibody is also immunoreactive with the same epitope of AgGM4.

By a "melanoma-associated" antigen is meant an antigen that is expressed to a greater extent in malignant melanoma cells than in normal or non-melanoma cells.

By the term "diagnostically effective" is meant that an amount of antibody is administered in sufficient quantity to enable detection of the site having the AgGM4 antigen for which the monoclonal antibody is specific.

By the term "effective amount" or "therapeutically effective amount" of the monoclonal antibody of the invention is meant an amount sufficient to obtain

the desired physiological effect, e.g., treatment of a malignant disease such as melanoma. An effective amount of the monoclonal antibody of the invention is generally determined by the physician in each case on the basis of factors normally considered by one skilled in the art to determine appropriate dosages, including the age, sex, and weight of the subject to be treated, the condition being treated, and the severity of the medical condition being treated.

The terms "treatment", "treating" and the like are used herein to generally mean obtaining a desired pharmacologic and/or physiologic effect. The effect may by prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. "Treatment" as used herein covers any treatment of a disease in a mammal, particularly a human, and includes:

(a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it;

(b) inhibiting the disease, i.e., arresting its development; or

(c) relieving or ameliorating the disease, i.e., causing regression of the disease. The invention is directed to treating patients with or at risk for development of melanoma. More specifically, "treatment " is intended to mean providing a therapeutically detectable and beneficial effect on a patient suffering from melanoma.

By "anti-idiotypic" antibody is meant an antibody which recognizes unique determinants present on an antibody of interest. These determinants are located in the hyper variable region of the antibody. It is this region which binds to a given epitope and, thus, is responsible for the specificity of the antibody. The anti-idiotypic antibody can be prepared by immunizing an animal with the antibody of interest. The immunized animal will recognize and respond to the idiotypic determinants of the immunizing antibody by producing an antibody to these idiotypic determinants. Hybridomas may be generated by methods known to those skilled in the art which produce an anti-idiotypic antibody (Herlyn et al. (1986)

Science 232:100). By using the anti-idiotypic antibodies of the second animal, which are specific for the antibody produced by a single hybridoma used to immunize the second animal, it is possible identify other clones with the same idiotype as the antibody of the hybridoma used for immunization. Idiotype identity between monoclonal antibodies of two hybridomas demonstrates that the two monoclonal antibodies are the same with respect for their recognition of the same epitopic determinant. Thus, by using antibodies to the epitopic determinants on a monoclonal antibody, it is possible to identify other hybridomas expressing monoclonal antibodies of the same epitopic specificity.

It is also possible to use the anti-idiotype technology to produce monoclonal antibodies which mimic an epitope. For example, an anti-idiotypic monoclonal antibody made to a first monoclonal antibody will have a binding domain in the hypervariable region which is the "image" of the epitope bound by the first monoclonal antibody. Thus, in this instance, the anti-idiotypic monoclonal antibody could be used for immunization since the anti-idiotype monoclonal antibody binding domain effectively acts as an antigen. When the monoclonal antibodies of the invention are used in the form of fragments, such as, for example, Fab and F(ab')2, and especially when these fragments are therapeutically labeled, any isotype can be used since amelioration of the malignancy in these situations is not dependent upon complement-mediated cytolytic destruction of those melanoma cells bearing the AgGM4 antigen.

The monoclonal antibodies of the invention can be used in any animal in which it is desirable to administer *in vitro* or *in vivo* immunodiagnosis or immunotherapy. The term "animal" as used herein is meant to include both humans as well as non-humans.

DNA sequences encoding the monoclonal antibody of the invention can be expressed *in vitro* by DNA transfer into a suitable host cell. "Host cells" are cells in which a vector can be propagated and its DNA expressed. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during

replication. However, such progeny are included when the term "host cell" is used. Methods of stable transfer, meaning that the foreign DNA is continuously maintained in the host, are known in the art.

In the present invention, the polynucleotide sequences of the monoclonal antibody of the invention may be inserted into a recombinant expression vector. The term "recombinant expression vector" refers to a plasmid, virus or other vehicle known in the art that has been manipulated by insertion or incorporation of the GM4-IgG4.λ genetic sequences. Such expression vectors contain a promoter sequence which facilitates the efficient transcription of the inserted genetic sequence of the host. The expression vector typically contains an origin of replication, a promoter, as well as specific genes which allow phenotypic selection of the transformed cells. Vectors suitable for use in the present invention include, but are not limited to the T7-based expression vector for expression in bacteria (Rosenberg et al. (1987) Gene 56:125), the pMSXND expression vector for expression in mammalian cells (Lee & Nathans (1988) J. Biol. Chem. 263:3521) and baculovirus-derived vectors for expression in insect cells. The DNA segment can be present in the vector operably linked to regulatory elements, for example, a promoter (e.g., T7, metallothionein 1, or polyhedrin promoters).

Polynucleotide sequences encoding the monoclonal antibody of the invention can be expressed in either prokaryotes or eukaryotes. Hosts can include microbial, yeast, insect and mammalian organisms. Methods of expressing DNA sequences having eukaryotic or viral sequences in prokaryotes are well known in the art.

Biologically functional viral and plasmid DNA vectors capable of expression and replication in a host are known in the art. Such vectors are used to incorporate DNA sequences of the invention.

Transformation of a host cell with recombinant DNA may be carried out by conventional techniques as are well known to those skilled in the art. Where the host is prokaryotic, such as *E. coli*, competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and

subsequently treated by the $CaCl_2$ method using procedures well known in the art. Alternatively, $MgCl_2$ or RbCl can be used.

Transformation can also be performed after forming a protoplast of the host cell if desired.

When the host is a eukaryote, such methods of transfection of DNA as calcium phosphate co-precipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors may be used. Eukaryotic cells can also be co-transformed with DNA sequences encoding the monoclonal antibody of the invention, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus, to transiently infect or transform eukaryotic cells and express the antibody (see, for example, Eukaryotic Viral Vectors, Cold Spring Harbor Laboratory, Gluzman ed., 1982).

Isolation and purification of microbial expressed polypeptide, or fragments thereof, provided by the invention, may be carried out by conventional means including preparative chromatography and immunological separations involving monoclonal or polyclonal antibodies.

The above descriptions for GM4-IgG.λ apply to AgGM4 as well.

Generation of a Monoclonal Antibody Specific for a Melanoma-Associated Antigen.

The strategies used in the generation of monoclonal antibodies (MAbs) can be greatly simplified with a preselection of the targeted immunized B lymphocyte. One recent approach has been through the use of magnetic beads (Kemmner et al. (1992) J. Immunol. Methods 147:197; Lea et al. (1988) J. Mol. Recognition 1:9; Sharma et al. (1993) Biotechniques 15:610) used to isolate rare and potentially useful reagents and cell types (Lundkvist et al. (1993) J. Gen. Virol. 74:1303; Ossendorp et al. (1989) J. Immunol. Methods 1210:191). The specificity of antibodies combined with the speed of magnetic separation has been a powerful

method for immunoselection. For human antibody development, magnetic bead immunoselection has resulted in potentially clinically useful reagents (Casali et al. (1986) Science 234:476. Lundkvist et al. (1993) supra demonstrated that a neutralizing human antibody to Puumala avirus can be obtained by using antigen-coated magnetic beads.

The present invention describes a method of using magnetic beads to enrich for subpopulations of human B lymphocytes having specifically desired functions. The IgG4 isotype was selected because although it is of the gamma class, it does not have any of the classical effector functions. For the imaging study described below, it was desired that the targeting antibody ideally not have any secondary effector functions, since such functions could interfere with the imaging process and perhaps alter the pharmacokinetics of the antibody. Another advantage of selecting an antibody having the IgG4 isotype is that this isotype is the rarest of the gamma isotypes and an enriched population facilities detection over background.

The presently described human monoclonal antibody is specific for a melanoma-associated antigen AgGM4. AgGM4 has a molecular weight of about 57 kD as determined by SDS-PAGE and specifically binds to GM4-IgG.λ. AgGM4 is a cell surface antigen that contains 27 C-terminal amino acids found in vimentin. Vimentin is a protein belonging to the class of intermediate filaments of the cell, which includes other proteins such as keratins, desmin, neurofilaments, and nuclear lamins (for a review, see, Lazarides (1982) Annu. Rev. Biochem. 51:219-250). It is present in the majority of cells of mesenchymal and nonmesenchymal origin, and its filaments are associated with both the nuclear and plasma membranes, however, vimentin is not a cell surface protein. The human vimentin gene has been cloned (Ferrari et al. (1986) Mol. Cell. Biol. 6:3614-3620). The C-terminal amino acids of vimentin (Ferrari, *et al.*, supra) also found in AgGM4 are shown in SEQ ID NO:5 as:

RTLLIKTVETRDGQVINETSQHHDDLE

The general method used for production of hybridomas secreting monoclonal antibodies is well known (Kohler & Milstein (1975) Nature 256:495). In the method described more fully below, pooled lymphocytes were obtained from cancer patients, enriched for heavy chain isotype IgG4, and hybridomas generated. The hybridomas were screened for production of an antibody to malignant cells lines and a cell line producing an antibody specific to a melanoma-related antigen isolated. The monoclonal antibody, termed GM4-IgG4.λ, was sequenced and the DNA sequences and predicted amino acid sequence of the light and heavy chains determined. The melanoma-associated antigen bound by GM4-IgG4.λ was isolated and sequenced. The antigen was identified as having a common amino acid sequence as found in the carboxy terminus of the intermediate filament, vimentin. The cDNA clone encoding the antigen contained vimentin-like sequences. The vimentin-like sequences have also been found to be assoicated with receptior like moleucles, i.e., V-FAM-1 and Thy-1 (Fuchs & Weber (1994) Annu. Rev. Biochem. 63:345-82).

The isolation of a tumor-specific antibody may also be performed without an enrichment step. The major advantage of immunoselection is the ability to enhance the chances of obtaining the cell type of choice. The results of the experiments described below show that specific antibodies of a certain rare isotype useful for clinical and therapeutic applications can be obtained from heterogeneous cell populations using simple, reproducible magnetic bead separation technology.

In one aspect, the invention is directed to hybridomas producing monoclonal antibodies specific to an antigen associated with a malignant disease. The isolation of hybridomas secreting monoclonal antibodies with the reactivity of the monoclonal antibodies of the invention can be accomplished using routine screening techniques to determine the elementary reaction pattern of the monoclonal antibody of interest. Thus, if a monoclonal antibody being tested binds with AgGM4 with the same specificity as the antibody of the present invention, then the antibody being tested and the antibody produced by the hybridomas of the invention are equivalent.

In the present invention, pooled lymphocytes isolated from cancer patients were either used as is or magnetically enriched for IgG4 (Example 2). FACS analysis showed that the unseparated, pooled lymphocytes were approximately 1.5% IgG4 positive, whereas the magnetically separated lymphocytes were approximately 20% IgG4 positive. In two separate fusion experiments with unseparated cells (Table 1, columns 1 & 2) only one clone out of 481 generated hybridomas (220 + 261), representing about 0.2%, had detectable IgG4 in the supernatant. In two separate fusions with magnetically separated cells, 21 clones out of 101 hybridomas, representing about 21%, had detectable IgG4 in the culture supernatant (Table 1, columns 3 & 4). The majority of the hybridomas from the unseparated cells secreted IgM (403 out of 481), whereas approximately half of the hybridomas from the separated cells secreted IgM (48 out of 101). The other IgG subclasses were similar in relative abundance regardless of whether the lymphocytes were separated or not.

Supernatant-containing antibody from the generated human hybridomas were screened against a panel of cell types, predominantly against melanoma and lung cells since this was the tissue origin of the pooled lymphocytes nodes. One IgG4-secreting human-human hybridoma, derived from fusion 3 (see Table 1) and designated GM4-IgG4.λ, showed unique specificity. This hybridoma was subcloned by limiting dilution (1 cell/3 wells) and the supernatant was rescreened against a larger panel of cell types (Table 2). Those cell types demonstrating the greatest GM4-IgG4.λ binding activity were melanoma (SK- mel-28), neuroblastoma (U87.MG), pancreas (Panc-1), and lung (Calu-1). The majority of the cell types tested were negative by enzyme immunoassay.

Flow cytometry analysis of GM4-IgG4.λ binding to cytoplasmic and surface melanoma antigens is shown in Table 3. Although the cytoplasmic reactivity of GM4-IgG4.λ was stronger with fixed cells, surface staining was also clearly present on living cells. Low density cultures (approximately 10% confluent) were started on day 0 and analyzed for antigen expression on subsequent days. As a function of *in vitro* culturing time, a decrease in the staining of surface

antigen-positive cells was observed with GM4-IgG4.λ and a concomitant increase in cytoplasmic staining (Table 3). Representative bindings of the GM4-IgG4.λ antibody to viable and fixed cells in 1 3, and 5 day old cultures are shown in Figs. 2A-2C. Beyond the third day of culture no surface staining was observed.

Flow cytometric analysis of 1 day old cultures of MENMEL94 cells to study the expression of antigen(s) in relationship to the DNA content showed that antigen was detected throughout all phases of the cell cycle. DNA histograms during the cell cycle showed a gradual increase in the number of cells in G_0/G_1 and a decrease of cells in S and G_2/M as cultures aged (Table 4). The accumulation of quiescent cells (G_0/G_1) may correspond with the absence of detectable surface antigen and the increased cytoplasmic uptake of human MAb in a large number of cells of old cultures.

Immunoreactivity of GM4-IgG4.λ with thin sections of tissue samples showed extensive binding to tumor cells. Little to no reactivity with normal cells and tissues was observed, with the exception of occasional light staining of melanin pigment-containing cells. Both cytoplasmic and membrane staining of tumor cells were observed as well in the tissue sections as well. Though the majority of the tumor cells were intensely stained, there were evident distinct subpopulations of cells which were moderately and lightly stained, indicative of heterogeneous antigen expression.

Antigen characterization. Extracts of HTB72 (SK-MEL-28) were analyzed by Western blot with GM4-IgG4.λ antibody (Example 3). The single major band with an apparent molecular weight of 57 kDa was observed. The fusion protein containing the AgGM4 epitope was purified and sequenced (see, SEQ ID NO:5). The antigen recognized by GM4-IgG4.λ contains a short stretch of amino acids also found in the carboxy-terminus of vimentin, namely the sequence RTLLIKTVETRDGQVINETSQHHDDLE (SEQ ID NO:5).

DNA and Amino Acid Sequence of GM4-IgG4.λ. DNA and derived amino acid sequences were determined for the light and heavy chains of the GM4-IgG4.λ antibody (Example 4) by standard methods. The DNA and derived amino acid sequences of the immunoglobulin light and heavy chain are shown in Figs. 3 and 4, respectively.

The specificity of GM4-IgG4.λ was compared to a commercially available anti-vimentin murine monoclonal antibody V6630 (Sigma). V6630 reacts the same in fixed and non-fixed cells on days 1 or 5, thus the expression of antigen in early or late log phase does not effect V6630 activity. V6630 had a high binding activity on KATO III cells, while no activity was detected for GM4-IgG4.λ (Table 8) with these cells, indicating that the GM4-IgG4.λ and V6630 antibodies recognize different epitopes on the AgGM4 antigen.

Diagnostic Uses

The monoclonal antibodies of the invention are suited for use, for example, in immunoassays in which they can be utilized in liquid phase or bound to a solid phase carrier. In addition, the monoclonal antibodies in these immunoassays can be detectably labeled in various ways. Examples of types of immunoassays which can utilize monoclonal antibodies of the invention are competitive and non-competitive immunoassays in either a direct or indirect format. Examples of such immunoassays are the radioimmunoassay (RIA) and the sandwich (immunometric) assay. Detection of the antigens using the monoclonal antibodies of the invention can be done utilizing immunoassays which are run in either the forward, reverse, or simultaneous modes, including immunohistochemical assays on physiological samples. Those of skill in the art will know, or can readily discern, other immunoassay formats without undue experimentation.

The monoclonal antibody of the invention is useful for detecting melanoma cells, since such cells have been shown to express an increased level of AgGM4 molecule on their cell surface. The monoclonal antibody of the invention is contacted with a source sample suspected of containing melanoma cells. The

amount of monoclonal antibody bound to the sample may be compared to the amount bound to a control source, e.g., a source known not to contain the AgGM4 antigen. Increased binding of the monoclonal antibody to the source relative to control is indicative of the presence of melanoma cells. Preferably, the monoclonal antibody binding is 25%-100% greater with a source containing melanoma cells than in the control; more preferably, the binding is 50%-100% greater; and most preferably, binding is 75%-100% greater.

The monoclonal antibodies of the invention can be bound to many different carriers and used to detect the presence of AgGM4. Examples of well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the invention. Those skilled in the art will know of other suitable carriers for binding monoclonal antibodies, or will be able to ascertain such, using routine experimentation.

There are many different labels and methods of labeling known to those of ordinary skill in the art. Examples of the types of labels which can be used in the present invention include enzymes, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds, and bio-luminescent compounds. Those of ordinary skill in the art will know of other suitable labels for binding to the monoclonal antibody, or will be able to ascertain such, using routine experimentation. Furthermore, the binding of these labels to the monoclonal antibody of the invention can be done using standard techniques common to those of ordinary skill in the art.

For purposes of the invention, AgGM4 may be detected by the monoclonal antibodies of the invention when present in biological fluids and tissues.

Any sample containing a detectable amount of AgGM4 can be used. A sample can be a liquid such as urine, saliva, cerebrospinal fluid, blood, serum and

the like, or a solid or semi-solid such as tissues, feces, and the like, or, alternatively, a solid tissue such as those commonly used in histological diagnosis.

Another technique which may also result in greater sensitivity consists of coupling the antibodies to low molecular weight haptens. These haptens can then be specifically detected by means of a second reaction. For example, it is common to use such haptens as biotin, which reacts with avidin, or dinitrophenyl, pyridoxal, and fluorescein, which can react with specific anti-hapten antibodies.

As used in this invention, the term "epitope" is meant to include any determinant capable of specific interaction with the monoclonal antibodies of the invention. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics.

In using the monoclonal antibodies of the invention for the *in vivo* detection of antigen, the detectably labeled monoclonal antibody is given in a dose which is diagnostically effective. The term "diagnostically effective" means that the amount of detectably labeled monoclonal antibody is administered in sufficient quantity to enable detection of the site having the AgGM4 antigen for which the monoclonal antibodies are specific.

The concentration of detectably labeled monoclonal antibody which is administered should be sufficient such that the binding to those cells having AgGM4 is detectable compared to the background. Further, it is desirable that the detectably labeled monoclonal antibody be rapidly cleared from the circulatory system in order to give the best target-to-background signal ratio.

As a rule, the dosage of detectably labeled monoclonal antibody for *in vivo* diagnosis will vary depending on such factors as age, sex, and extent of disease of the individual. The dosage of monoclonal antibody can vary from about 0.01 mg/m^2 to about 500 mg/m^2, preferably 0.1 mg/m^2 to about 200 mg/m^2, most preferably about 0.1 mg/m^2 to about 10 mg/m^2. Such dosages may vary, for

example, depending on whether multiple injections are given, tumor burden, and other factors known to those of skill in the art.

For *in vivo* diagnostic imaging, the type of detection instrument available is a major factor in selecting a given radioisotope. The radioisotope chosen must have a type of decay which is detectable for a given type of instrument. Still another important factor in selecting a radioisotope for *in vivo* diagnosis is that the half-life of the radioisotope be long enough so that it is still detectable at the time of maximum uptake by the target, but short enough so that deleterious radiation with respect to the host is minimized.

Ideally, a radioisotope used for *in vivo* imaging will lack a particle emission, but produce a large number of photons in the 140-250 keV range, which may be readily detected by conventional gamma cameras.

For *in vivo* diagnosis radioisotopes may be bound to immunoglobulin either directly or indirectly by using an intermediate functional group. Intermediate functional groups which often are used to bind radioisotopes which exist as metallic ions to immunoglobulins are the bifunctional chelating agents such as diethylene triaminepentacetic acid (DTPA) and ethylenediaminetetraacetic acid (EDTA) and similar molecules. Typical examples of metallic ions which can be bound to the monoclonal antibodies of the invention are ^{111}In, ^{97}Ru, ^{67}Ga, ^{68}Ga, ^{72}As, ^{89}Zr, ^{90}Y, and ^{201}Tl.

The monoclonal antibodies of the invention can also be labeled with a paramagnetic isotope for purposes of *in vivo* diagnosis, as in magnetic resonance imaging (MRI) or electron spin resonance (ESR). In general, any conventional method for visualizing diagnostic imaging can be utilized.

Usually gamma and positron emitting radioisotopes are used for camera imaging and paramagnetic isotopes for MRI. Elements which are particularly useful in such techniques include ^{157}Gd, ^{55}Mn, ^{162}Dy, ^{52}Cr, and ^{56}Fe.

The monoclonal antibodies of the invention can be used to monitor the course of amelioration of malignancy in an animal. Thus, by measuring the increase or decrease in the number of cells expressing AgGM4 or changes in

the concentration of AgGM4 present in various body fluids, it would be possible to determine whether a particular therapeutic regimen aimed at ameliorating the malignancy is effective.

Therapeutic Uses

The term "ameliorate" denotes a lessening of the detrimental affect of the malignancy in the animal receiving therapy. The term "therapeutically effective" means that the amount of monoclonal antibody used is of sufficient quantity to ameliorate the malignancy.

The monoclonal antibodies of the invention can also be used, alone or in combination with effector cells (Douillard et al. (1986) Hybridoma 5 (Supp. 1:S139), for immunotherapy in an animal having a cells which expresses AgGM4 antigen with epitopes reactive with the monoclonal antibodies of the invention.

When used for immunotherapy, the monoclonal antibodies of the invention may be unlabeled or labeled with a therapeutic agent. These agents can be coupled either directly or indirectly to the monoclonal antibodies of the invention. One example of indirect coupling is by use of a spacer moiety. These spacer moieties, in turn, can be either insoluble or soluble (Diener et al. (1986) Science 231:148) and can be selected to enable drug release from the monoclonal antibody molecule at the target site. Examples of therapeutic agents which can be coupled to the monoclonal antibodies of the invention for immunotherapy are drugs, radioisotopes, lectins, and toxins.

The drugs with which can be conjugated to the monoclonal antibodies of the invention include non-proteinaceous as well as proteinaceous drugs.

The terms "non-proteinaceous drugs" encompasses compounds which are classically referred to as drugs, for example, mitomycin C, daunorubicin, and vinblastine.

The proteinaceous drugs with which the monoclonal antibodies of the invention can be labeled include immunomodulators and other biological response modifiers. The term "biological response modifiers" is meant to encompass

substances which are involved in modifying the immune response in such manner as to enhance the destruction of the antigen-bearing malignant cell or tumor for which the monoclonal antibodies of the invention are specific. Examples of immune response modifiers include such compounds as lymphokines. Lymphokines include tumor necrosis factor, interleukins 1, 2, and 3, lymphotoxin, macrophage activating factor, migration inhibition factor, colony stimulating factor, and interferon. Interferons with which the monoclonal antibodies of the invention can be labeled include alpha-interferon, beta-interferon, and gamma-interferon and their subtypes.

In using radioisotopically conjugated monoclonal antibodies of the invention for immunotherapy certain isotypes may be more preferable than others depending on such factors as leukocyte distribution as well as isotype stability and emission. If desired, the malignant cell distribution can be evaluated by the *in vivo* diagnostic techniques described above. Depending on the malignancy some emitters may be preferable to others. In general, alpha and beta particle-emitting radioisotopes are preferred in immunotherapy. For example, if an animal has solid tumor foci, as in a carcinoma, a high energy beta emitter capable of penetrating several millimeters of tissue, such as ^{90}Y, may be preferable. On the other hand, if the malignancy consists of simple target cells, as in the case of leukemia, a short range, high energy alpha emitter, such as ^{212}Bi, may be preferable. Examples of radioisotopes which can be bound to the monoclonal antibodies of the invention for therapeutic purposes are ^{125}I, ^{131}I, ^{90}Y, ^{67}Cu, ^{212}Bi, ^{211}At, ^{212}Pb, ^{47}Sc, ^{109}Pd, and ^{188}Re.

Lectins are proteins, usually isolated from plant material, which bind to specific sugar moieties. Many lectins are also able to agglutinate cells and stimulate lymphocytes. However, ricin is a toxic lectin which has been used immunotherapeutically. This is preferably accomplished by binding the alpha-peptide chain of ricin, which is responsible for toxicity, to the antibody molecule to enable site specific delivery of the toxic effect.

Toxins are poisonous substances produced by plants, animals, or microorganisms that, in sufficient dose, are often lethal. Diphtheria toxin is a

substance produced by *Corynebacterium diphtheria* which can be used therapeutically. This toxin consists of an alpha and beta subunit which under proper conditions can be separated. The toxic A component can be bound to an antibody and used for site specific delivery to a AgGM4-bearing cell for which the monoclonal antibodies of the invention are specific. Other therapeutic agents which can be coupled to the monoclonal antibodies of the invention are known, or can be easily ascertained, by those of ordinary skill in the art.

The labeled or unlabeled monoclonal antibodies of the invention can also be used in combination with therapeutic agents such as those described above. Especially preferred are therapeutic combinations comprising the monoclonal antibody of the invention and immunomodulators and other biological response modifiers.

Thus, for example, the monoclonal antibodies of the invention can be used in combination with alpha-interferon. This treatment modality enhances monoclonal antibody targeting of melanomas by increasing the expression of monoclonal antibody reactive antigen by the melanoma cells (Greiner et al. (1987) Science 235:895). Alternatively, the monoclonal antibody of the invention could be used, for example, in combination with gamma-interferon to thereby activate and increase the expression of Fc receptors by effector cells which, in turn, results in an enhanced binding of the monoclonal antibody to the effector cell and killing of target malignant cells. Those of skill in the art will be able to select from the various biological response modifiers to create a desired effector function which enhances the efficacy of the monoclonal antibody of the invention.

When the monoclonal antibody of the invention is used in combination with various therapeutic agents, such as those described herein, the administration of the monoclonal antibody and the therapeutic agent usually occurs substantially contemporaneously. The term "substantially contemporaneously" means that the monoclonal antibody and the therapeutic agent are administered reasonably close together with respect to time. Usually, it is preferred to administer the therapeutic agent before the monoclonal antibody. For example, the therapeutic agent can be

administered 1 to 6 days before the monoclonal antibody. The administration of the therapeutic agent can be daily, or at any other interval, depending upon such factors, for example, as the nature of the malignancy, the condition of the patient and half-life of the agent.

Using the monoclonal antibodies of the invention, it is possible to design therapies combining all of the characteristics described herein. For example, in a given situation it may be desirable to administer a therapeutic agent, or agents, prior to the administration of the monoclonal antibodies of the invention in combination with effector cells and the same, or different, therapeutic agent or agents. For example, it may be desirable to treat patients with malignant disease by first administering gamma-interferon and interleukin-2 daily for 3 to 5 days, and on day 5 administer the monoclonal antibody of the invention in combination with effector cells as well as gamma-interferon, and interleukin-2.

It is also possible to utilize liposomes with the monoclonal antibodies of the invention in their membrane to specifically deliver the liposome to the area of the tumor or cells expressing AgGM4. These liposomes can be produced such that they contain, in addition to the monoclonal antibody, such immunotherapeutic agents as those described above which would then be released at the site of malignancy (Wolff et al. (1984) Biochem. Biophys. Acta 802:259).

The dosage ranges for the administration of the monoclonal antibodies of the invention are those large enough to produce the desired effect in which the symptoms of the malignant disease are ameliorated. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of the disease in the patient and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician in the event of any complication. Dosage can vary from about 0.1 mg/kg to about 2000 mg/kg, preferably about 0.1 mg/kg to about 500 mg/kg, in one or more dose administrations daily, for one or several days. Generally, when the monoclonal antibodies of the invention are administered conjugated with therapeutic agents

lower dosages, comparable to those used for *in vivo* immunodiagnostic imaging, can be used.

The monoclonal antibodies of the invention can be administered parenterally by injection or by gradual perfusion over time. The monoclonal antibodies of the invention can be administered intravenously, intraperitoneally, intra-muscularly, subcutaneously, intracavity, or transdermally, alone or in combination with effector cells.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

The invention also relates to a method for preparing a medicament or pharmaceutical composition comprising the monoclonal antibodies of the invention, the medicament being used for therapy of malignant disorders.

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use various constructs and perform the various methods of the present invention and are not intended to limit the scope of what the inventors regard as their invention. Unless indicated otherwise, parts are parts by weight, temperature is in degrees centigrade, and pressure is at or near atmospheric pressure. Efforts have been made to ensure accuracy with respect to numbers used, (e.g., length of DNA

sequences, molecular weights, amounts, particular components, etc.) but some deviations should be accounted for.

Example 1. Cells and Cell Lines

Cell Lines. The following human cell lines were used: melanoma cell lines HTB63, HTB64, HTB66, HTB69, HTB70, HTB71, HTB72, HTB73, A375, M21, M14, HH211, and HH443; kidney carcinoma cell line HTB46; colon carcinoma cell lines CaCo2, Colo 205, HT29; neuroblastoma cell lines LAN-1, U87-MG; ovarian cancer cell lines HTB75, HTB77; lymphocyte cell lines Daudi, WIL-2, Molt-4; prostate carcinoma cell line PC-3; gastric carcinoma cell line Kato-III; pancreatic cancer cell line Panc-1; lung carcinoma cell lines A375, Calu-1, SK-MES-1, UCLA-P3, NCIH661; breast carcinoma cell line SK-Br-3; primary metastatic melanoma tumor cells MENMEL94; normal fibroblast WI-38; and human fusion partner SHFP-1 (Glassy (1989) J. Tissue Cult. Methods 12:85). With the exception of MENMEL94, HH211, and HH443 and SHFP-1, all cell lines were obtained from the American Type Culture Collection (Rockville, MD). All cells and generated hybridomas were grown in RPMI-1640 medium supplemented with 10% FCS and 2mM glutamine in a humidified 37 C, 5% C02/95% air incubator. MENMEL94 was cultured in McCoy's medium supplemented with 10% FCS, 2 mM glutamine, antibiotic-antimycotic, MEM vitamins, and 0.05 mM non-essential amino acids. Cell viability was determined using 0.05% trypan blue.

Example 2. Isolation of a GM4-Specific Human Monoclonal Antibody.

Briefly, the ampules of cryopreserved cells containing the B-cell lymphocytes from cancer patients were thawed and resuspended at 5 x 10^6/ml in RPMI-1640 medium supplemented with 10% FCS and 2 mM glutamine and incubated overnight at 37°C in 5% CO^2/95% air prior to fusion.

MAb-directed, B cell enhancement protocol. The pooled lymphocytes (5 x 10^6 cells/ml) were mixed with biotinylated SC-HuG4 (1:100 dilution; 500

ng/ml), a murine MAb specific to the human IgG4 isotype and gently agitated in an incubator (37°C) for one hour.

After 3x washing, the antibody coated lymphocytes were mixed with streptavidin-conjugated magnetic beads (Dynabead M-280) at a ration of 3 beads per cell and incubated for 30 min. The cell-bead suspension was then diluted with RPMI-1640 + 10% FCS to 1 x 10^5 cells/ml and separated with the Dynal MPC-1 (magnetic particle concentrator) according to manufacturer's instructions, followed by trypsinization of the cell/bead complex for harvesting cells.

Fusion, cloning, and characteristics of hybridomas. Details of the fusion and cloning of human-human hybridomas generated from SHFP-1 and pooled lymphocytes have been previously described (Glassy (1987) supra; Glassy (1989) supra). Table 1 summarizes the generation of human-human hybridomas resulting from somatic cell fusions between the pooled lymphocytes and SHFP-1 cells. Four separate fusion experiments were performed. Fusion 1 (generating 220 IgG4+ hybridomas) and Fusion 2 (generating 261 IgG4+ hybridomas) were performed with unseparated lymphocytes. Fusion 3 (generating 40 IgG4+ hybridomas) and Fusion 4 (generating 61 IgG4+ hybridomas) were performed with magnetically separated cells. Each isotype is represented by the number of IgG4 positives over the total number of Ig positive hybridomas generated in each fusion, followed by the relative percent.

Enzyme immunoassay. Quantitation and qualitation of mouse and human antibodies were assessed by an enzyme immunoassay previously described (Glassy et al. (1983) J. Immunol. Methods 58:119) and modified as described (Glassy & Surh (1985) J. Immunol. Methods 81:115; Glassy & Cleveland (1985) Meth. Enzymol. 121:525, both of which publications are herein specifically incorporated by reference).

Immunoreactive human monoclonal antibodies were identified by an indirect EIA to panels of cell lines immobilized on Immunon I (Dynatech) micro-EIA plates.

All human MAbs which bind with greater than 2 times the absorbance of an irrelevant control antibody to a relevant tumor cell line are assumed positive

in preliminary tests. The strategies used in screening for tumor-reactive human MAbs have been previously described (Gaffar et al. (1986) BioEssays 4:119; Glassy (1993) supra).

Immunofluorescence and FACS analysis. Reactivity of the human MAb SC-GM4 with cell surface antigen(s) was assessed by indirect immunofluorescence and flow cytometry as described (Chang et al. (1994) Ann. Surg. Oncol. 1:213). Briefly, menastatic MENMEL94 tumor cells were harvested by 0.2% ethylenediamine tetraacetic acid (EDTA) or Ca^{++}, Mg^{++}-free PBS, washed and incubated with 50, 20, 10, or 4 ug of SC-GM4 for 4 hr at 4°C. Post incubation, cells were washed once in PBS and treated with fluorescein isothiocyanate labelled goat anti-human IgG antibody for another 30 min. Reactivity of the MAb was determined by analyzing 10,000 cells with an Argon laser or a FACScan (Becton Dickinson, Inc.). Unstained control cells, as well as FITC positive cells were stained with 25 ug propidium iodide for 5 min in PBS so as to gate live cells. After collection, negative markers were set to include 99.9% of the dead cells.

Cells falling outside these markers were considered as live cells. Reactivity of GM4 with unrelated cell lines SKBR-3 (breast) and CALU-1 (lung), as well as reactivity of MENMEL94 with isotype matched IgG antibody (Sigma) was used as control. Results are shown in Table 3.

DNA analysis. DNA cell cycle was analyzed on 1, 3, and 5 days old cultures of MENMEL94 by using the Cycle Test Plus kit (Becton Dickinson). Briefly, 5 x 10^5 cells were suspended in 1.12% sodium citrate buffer, centrifuged at 300x g and washed twice. These cells were then trypsinized for 10 min and 200 ul of 1.12% sodium citrate buffer containing spermine tetrahydrochloride, RNase, trypsin inhibitor added. The cells were further incubated for 10 min at room temperature, then stained for 10 min in 10 ug propidium iodide. Cellular DNA content was analyzed within 3 hr by flow cytometry as described above using Cell FIT software (Dean (1987) in: Techniques in Cell Cycle Research (J. Gray & Z. Darzynkiewicz, eds.); Humana Press, Clifton, NJ; pp. 207-253). Results are shown

in Table 4. Cell cycle determination of proportion of MENMEL94 cells in different phases (G_0/G_1, S, G_2/M) was performed using sum of broadened rectangles (SOBR; Dean (1987) supra).

Immunohistology. The reactivity of SC-GM4 was measured on surgical specimens of melanoma tissue as well as tumor cells grown on chamber slides by the indirect antibody peroxidase technique. Paraffin embedded tissue sections were cut 5 microns thick, deparaffinized in xylene and rehydrated in graded ethanol. Endogenous peroxidase activity was blocked by the addition of hydrogen peroxide in methanol (0.3% v/v) for 15 min. The primary antibody was added at a concentration of 0.1 mg/ml in PBS containing 1% (w/v) BSA to the cells for 2 hr. Goat anti-human biotinylated-IgG (Vectastain Elite ABC kit, Vector Laboratories, Inc., Burlingame, California) was added after sequential washings with PBS. The staining reaction was developed in diaminobenzidine, followed by counterstaining with Richard Allen hematoxylin and mounting in glycerol-gelatin (Guesdon et al. (1979) J. Histochem. Cytochem. 27:1131; Viale et al. (1989) J. Immunol 143:4338). Negative controls consisted of normal skin tissue from control subjects stained with the SC-GM4 antibody, melanoma tumor cells stained with irrelevant human IgG, as well as NCIH661 stained with SC-GM4. The results are shown in Table 2. The reactivity values were measured as O.D. 490 units: 0.00-0.150, "-"; 0.151-0.3, "+"; 0.3-0.6, "++";).6-0.9, "+++"; >0.9, "++++".

Example 3. Antigen Characterization.

Western Blot analysis. Western blot analysis was performed as described (Chang et al. (1994) supra). The single major band with an apparent molecular weight of 57 kDa was observed.

Example 4. Cloning of GM4-IgG4.λ and Comparison to Known Murine Vimentin Antibody V6630

EXPERIMENTAL DETAILS

The DNA and amino acid sequences of the light and heavy immunoglobulin chains of GM4-IgG4.λ are shown in Figs. 3 and 4, respectively.

Using fixed and fresh early passage metastic melanoma cells, it was shown that the GM4-IgG4.λ antibody has a high surface binding. FACS histogram analysis confirmed that the GM4-IgG4.λ antibody reacted very differently with early passage differentiated melanoma cells (HH211) relative to early passage undifferentiated melanoma cells (HH443) (Table 5). The reactivity of recombinant GM4-IgG4.λ antibody was weaker than the antibody produced by the hybridoma (Table 6). This may result from a lower concentration of antibody.

The binding activity of a commercial mouse monoclonal antibody specific for vimentin, V6630 (Sigma), was compared to that of the hybridoma and recombinant GM4-IgG4.λ antibodies (Tables 7 and 8). V6630 showed a different staining pattern than AgGM4 antibody. AgGM4 is a cell surface antigen while vimentin is not.

The instant invention is shown and described herein in what is considered to be the most practical, and preferred embodiments. It is recognized, however, that departures may be made therefrom which are within the scope of the invention, and that modifications will occur to one skilled in the art upon reading this disclosure.

Table 1. Lymphocyte x SHFP-1 fusion data summary.

Isotype	Fusion 1	Fusion 2	Fusion 3	Fusion 4
IgM	187/220 (83%)	216/261 (82%)	19-40 (47%)	29/61 (47%)
IgG1	25/220 (11%)	34/261 (13%)	8/40 (20%)	14/61 (23%)
IgG2	4/220 (2%)	1/261 (0.4%)	0/40 (0%)	4/61 (6%)
IgG3	3/220 (3%)	10/261 (4%)	3/40 (7%)	3/61 (5%)
IgG4	1/220 (0.5%)	0/261 (0%)	10/40 (25%)	11/61 (18%)

Table 2. EIA Reactivity Profile of GM4 with a Panel of Cell Types

Cell Type	Reactivity	Cell Type	Reactivity
Melanoma		Kidney	
HTB 63	+++	HTB 46	-
HTB 64	++++	Colon	
HTB 66	++++	CaCo2	-
HTB 69	-	Colo 205	-
HTB 70	++++	Ovary	
HTB 71	+++	HTB75	-
HTB 72	++++	HTB 77	-
HTB 73	-	Lymphocyte	
M14	-	Daudi	
M21	++	WIL-2	-
A375	++++	Molt-4	-
HH443	++++	Prostate	
HH211	++++	PC-3	-
MENMEL94	++++	Breast	
Lung		SK-Br-3	-
Calu-1	++++	Fibroblast	
SK-MES-1	+++	WI-38	-
UCLA-P3	-	Stomach	
NCIH661	-	Kato-III	-
Neuroblastoma			
LAN-1	-		
U87.MG	++++		
Pancreas			
Panc-1	++++		

Table 3. Progressive loss of surface AgGM4 in nonproliferating cells.

% Ag-GM4 Positive Cells						
	Day 1		Day 3		Day 5	
Cells	Cyto	Surface	Cyto	Surface	Cyto	Surface
MENMEL94	29	71	75	25	100	0

Table 4. Cell cycle analysis of MENMEL94 cells during culture.

Day	% G_0/G_1	% S	% G_2/M
1	61.7	28.8	9.5
3	72.0	21.5	6.3
5	73.9	19.1	7.0

Table 5. Reactivity of Hybridoma GM4-IgG4.λ MAb to Metastic Melanoma Cell Lines.

Cell Lines		Early Log Phase (Day 1)	Late Log Phase (Day 5)
Differentiated HH 211	cytoplasmic	nt	4%
	surface	nt	96%
Undifferentiated HH 443	cytoplasmic	46%	58%
	surface	54%	42%

nt=not tested

Table 6. Reactivity of Recombinant GM4-IgG4.λ MAb to Metastic Melanoma Cell Lines.

Cell Lines		Early Log Phase (Day 1)	Late Log Phase (Day 5)
Differentiated HH 211	cytoplasmic	60%	78%
	surface	40%	22%
Undifferentiated HH 443	cytoplasmic	29%	86%
	surface	71%	14%

Table 7. Reactivity of Murine V6630 MAb to Metastic Melanoma Cell Lines.

Cell Lines		Early Log Phase (Day 1)	Late Log Phase (Day 5)
Differentiated HH 211	cytoplasmic	4%	8%
	surface	96%	92%
Undifferentiated HH 443	cytoplasmic	19%	15%
	surface	81%	85%

Table 8. Reactivity of Anti-GM4 MAb and V6630 to Squamous Cell Carcinoma (KATO III).

MAb	Early Log Phase (Day 1)		Late Log Phase (Day 5)	
	Cytoplasmic	Surface	Cytoplasmic	Surface
GM4-IgG4.K	0%	0%	0%	0%
rGM4-IgG4.K	0%	0%	0%	0%
V6630	75%	25%	93%	7%

r=recombinant

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Novopharm Biotech, Inc. .

(ii) TITLE OF INVENTION: HUMAN MONOCLONAL ANTIBODY SPECIFIC FOR MELANOMA-ASSOCIATED ANTIGEN AND METHODS OF USE

(iii) NUMBER OF SEQUENCES: 5

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Fish & Richardson P.C.
(B) STREET: 4225 Executive Square, Suite 1400
(C) CITY: La Jolla
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 92037

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE: 23-APR-1996
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Haile Ph.D., Lisa A.,
(B) REGISTRATION NUMBER: 38,347
(C) REFERENCE/DOCKET NUMBER: 07244/005WO1

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (619) 678-5070
(B) TELEFAX: (619) 678-5099

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 387 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..387

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Met Ala Trp Thr Pro Leu Trp Leu Pro Leu Leu Thr Phe Cys Ser Val
 1               5                  10                  15

TCT GAG GCC TCC TAT GAG CTG ACA CAG CCA CCC TCG GTG TCA GTG TCC      96
Ser Glu Ala Ser Tyr Glu Leu Thr Gln Pro Pro Ser Val Ser Val Ser
            20                  25                  30

CCA GGA CAA ACG GCC AGG ATC ACC TGC TCT GGA GAT GCA TTG CCA GAA     144
Pro Gly Gln Thr Ala Arg Ile Thr Cys Ser Gly Asp Ala Leu Pro Glu
        35                  40                  45

AAA TAT GTT TAT TGG TAC CAG CAG AAG TCA GGC CAG GGC CCT GTG GTG     192
Lys Tyr Val Tyr Trp Tyr Gln Gln Lys Ser Gly Gln Gly Pro Val Val
    50                  55                  60

GTC ATC TAT GAG GAC AGC AAA CGA CCC TCC GGG ATC CCT GAG AGA TTC     240
Val Ile Tyr Glu Asp Ser Lys Arg Pro Ser Gly Ile Pro Glu Arg Phe
65                  70                  75                  80

TCT GGC TCC AGC TCA GGG ACA ATG GCC ACC TTG ACT ATC AGT GGG GCC     288
Ser Gly Ser Ser Ser Gly Thr Met Ala Thr Leu Thr Ile Ser Gly Ala
                85                  90                  95

CAG GTG GAA GAT GAA GGT GAC TAC TAC TGT TAC TCA ACT GAC AGC AGT     336
Gln Val Glu Asp Glu Gly Asp Tyr Tyr Cys Tyr Ser Thr Asp Ser Ser
            100                 105                 110

GGT TAT CAT AGG GAG GTG TTC GGC GGA GGG ACC AGG CTG ACC GTC CTA     384
Gly Tyr His Arg Glu Val Phe Gly Gly Gly Thr Arg Leu Thr Val Leu
        115                 120                 125

GGT                                                                 387
Gly
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 129 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Trp Thr Pro Leu Trp Leu Pro Leu Leu Thr Phe Cys Ser Val
1 5 10 15

Ser Glu Ala Ser Tyr Glu Leu Thr Gln Pro Pro Ser Val Ser Val Ser
20 25 30

Pro Gly Gln Thr Ala Arg Ile Thr Cys Ser Gly Asp Ala Leu Pro Glu
35 40 45

Lys Tyr Val Tyr Trp Tyr Gln Gln Lys Ser Gly Gln Gly Pro Val Val
50 55 60

Val Ile Tyr Glu Asp Ser Lys Arg Pro Ser Gly Ile Pro Glu Arg Phe
65 70 75 80

Ser Gly Ser Ser Ser Gly Thr Met Ala Thr Leu Thr Ile Ser Gly Ala
85 90 95

Gln Val Glu Asp Glu Gly Asp Tyr Tyr Cys Tyr Ser Thr Asp Ser Ser
100 105 110

Gly Tyr His Arg Glu Val Phe Gly Gly Gly Thr Arg Leu Thr Val Leu
115 120 125

Gly

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 432 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..432

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATG GAG TTT GGG CTG AGC TGG GTT TTC CTT GCT GCT ATT TTA AAA GGT 48
Met Glu Phe Gly Leu Ser Trp Val Phe Leu Ala Ala Ile Leu Lys Gly
130 135 140 145

GTC CAG TGT GAG GTG CAG CTC GAG GAG TCG GGG GGA GGC TTG GTA AAG 96
Val Gln Cys Glu Val Gln Leu Glu Glu Ser Gly Gly Gly Leu Val Lys
150 155 160

CCG GGG GGG TCC CTT AGA GTC TCC TGT GCA GCC TCT GGA TTC ACT TTC 144
Pro Gly Gly Ser Leu Arg Val Ser Cys Ala Ala Ser Gly Phe Thr Phe
165 170 175

AGA AAC GCC TGG ATG AGC TGG GTC CGC CAG GCT CCA GGG AAG GGG CTG 192
Arg Asn Ala Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
180 185 190

GAG TGG GTC GGC CGT ATT AAA AGC AAA ATT GAT GGT GGG ACA ACA GAC 240
Glu Trp Val Gly Arg Ile Lys Ser Lys Ile Asp Gly Gly Thr Thr Asp
195 200 205

TAT CCT GCA CCC GTG AAA GGC AGA TTC ACC ATC TCA AGA GAT GAT TCA 288
Tyr Pro Ala Pro Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser
210 215 220 225

AAA AAC ACG TTA TAT CTG CAA ATG AAT AGC CTG AAA GCC GAG GAC ACA 336
Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Lys Ala Glu Asp Thr
230 235 240

GCC GTA TAT TAC TGT ACC ACG GGG ATT ATG ATA ACA TTT GGG GGA GTT 384
Ala Val Tyr Tyr Cys Thr Thr Gly Ile Met Ile Thr Phe Gly Gly Val
245 250 255

ATC CCT CCC CCG AAT TGG GGC CAG GGA ACC CTG GTC ACC GTC TCC TCA 432
Ile Pro Pro Pro Asn Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
260 265 270

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 144 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Glu Phe Gly Leu Ser Trp Val Phe Leu Ala Ala Ile Leu Lys Gly
1 5 10 15

Val Gln Cys Glu Val Gln Leu Glu Glu Ser Gly Gly Gly Leu Val Lys
20 25 30

Pro Gly Gly Ser Leu Arg Val Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45

Arg Asn Ala Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
50 55 60

Glu Trp Val Gly Arg Ile Lys Ser Lys Ile Asp Gly Gly Thr Thr Asp
65 70 75 80

Tyr Pro Ala Pro Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser
85 90 95

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Lys Ala Glu Asp Thr
100 105 110

Ala Val Tyr Tyr Cys Thr Thr Gly Ile Met Ile Thr Phe Gly Gly Val
115 120 125

Ile Pro Pro Pro Asn Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
130 135 140

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..27

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Arg Thr Leu Leu Ile Lys Thr Val Glu Thr Arg Asp Gly Gln Val Ile
1 5 10 15

Asn Glu Thr Ser Gln His His Asp Asp Leu Glu
20 25

What is claimed is:

1. A human monoclonal antibody, wherein said antibody binds to AgGM4 antigen.

2. The monoclonal antibody of claim 1, said antibody having a light chain encoded by the DNA sequence of SEQ ID NO:1 and a heavy chain encoded by the DNA sequence of SEQ ID NO:3.

3. The monoclonal antibody of claim 1, wherein said light chain comprises the amino acid sequence of SEQ ID NO:2.

4. The monoclonal antibody of claim 1, wherein said heavy chain comprises the amino acid sequence of SEQ ID NO:4.

5. A continuous hybridoma cell line which secretes a monoclonal antibody having a light chain encoded by the DNA sequence of SEQ ID NO:1 and a heavy chain encoded by the DNA sequence of SEQ ID NO:3.

6. The hybridoma cell line of claim 5, wherein said light chain comprises the amino acid sequence of SEQ ID NO:2 and said heavy chain comprises the amino acid sequence of SEQ ID NO:4.

7. A method of detecting melanoma, comprising:
contacting a sample suspected of containing melanoma cells with a diagnostically effective amount of detectably labeled antibody or fragment thereof, wherein the antibody specifically binds to AgGM4; and
determining whether the antibody binds AgGM4,
wherein increased binding of said antibody to AgGM4 relative to a control is indicative of melanoma.

8. The method of claim 7, wherein said detecting is *in vivo*.

9. The method of claim 8, wherein the detectable label is selected from the group consisting of a radioisotope and a paramagnetic label.

10. The method of claim 7, wherein said detecting is *in vitro*.

11. The method of claim 10, wherein the detectable label is selected from the group consisting of a radioisotope, a fluorescent compound, a colloidal metal, a chemiluminescent compound, a bioluminescent compound, and an enzyme.

12. The method of claim 7, said antibody having a light chain encoded by the DNA sequence of SEQ ID NO:1 and a heavy chain encoded by the DNA sequence of SEQ ID NO:3.

13. A method of treating a malignant disease in an animal comprising administering to said animal a therapeutically effective amount of a monoclonal antibody or fragment thereof, wherein said antibody specifically binds AgGM4.

14. The method of claim 13, wherein said malignant disease is melanoma.

15. The method of claim 13, wherein said antibody is human.

16. The method of claim 13, wherein said administering is parenteral.

17. The method of claim 16, wherein said parenteral administration is by subcutaneous, intramuscular, intraperitoneal, intracavity, transdermal, or intraveous injection.

18. The method of claim 13, wherein the administration is at a dosage of about 0.01 mg/kg to about 2000 mg/kg/dose.

19. The method of claim 13, wherein the antibody is administered in combination with effector cells.

20. The method of claim 13, wherein the monoclonal antibody is therapeutically labeled.

21. The method of claim 20, wherein the therapeutic label is selected from the group consisting of a radioisotope, a drug, an immunomodulator, a biological response modifier, a lectin, and a toxin.

22. The method of claim 13, wherein the antibody is administered substantially contemporaneously in combination with a therapeutic agent.

23. The method of claim 22, wherein the therapeutic agent is selected from the group consisting of a radioisotope, a drug, an immunomodulator, a biological response modifier, a lectin, and a toxin.

24. The method of claim 13, said antibody having a light chain encoded by the DNA sequence of SEQ ID NO:1 and a heavy chain encoded by the DNA sequence of SEQ ID NO:3.

25. An anti-idiotypic antibody to the antibody of claim 1.

26. A method of treating malignant disease in an animal comprising administering said animal with an immunogenically effective amount of the anti-idiotypic antibody of claim 25.

27. A pharmaceutical composition comprising a therapeutically effective amount of the monoclonal antibody of claim 1, together with a pharmacological carrier.

28. A pharmaceutical composition comprising a therapeutically effective amount of the monoclonal antibody of claim 27, together with a pharmacological carrier.

29. Substantially purified AgGM4 antigen.

30. The antigen of claim 29, wherein the antigen is specifically bound by GM4-IgG4.λ.

31. The antigen of claim 29, wherein the antigen has a molecular weight of about 57 kD as determined by SDS-PAGE and is detectable on the cell surface of a melanoma cell.

32. The monoclonal antibody of claim 1, wherein the antibody is designated GM4-IgG4.λ or an antibody having the antigenic specificity of GM4-IgG4.λ.

33. An antibody producing cell and the progeny thereof that produce the antibody of claim 32.

34. An antibody producing cell having all the identifying characteristics of a cell according to claim 33.

35. A purified antibody having identifying characteristics identical to antibody produced by a cell according to claim 33.

36. A polynucleotide comprising a sequence encoding a polypeptide with immunological activity of monoclonal antibody GM4-IgG4.λ, wherein the polypeptide comprises at least 5 consecutive amino acids from a variable region of monoclonal antibody GM4-IgG4.λ.

37. A polynucleotide according to claim 36, wherein the variable region is from a light chain.

38. A polynucleotide according to claim 36, wherein the variable region is from a heavy chain.

39. The polynucleotide of claim 36, wherein the 5 consecutive amino acids is contained in SEQ. ID NO:2.

40. The polynucleotide of claim 36, wherein the 5 consecutive amino acids is contained in SEQ. ID NO:4.

41. The polynucleotide of claim 36, wherein the encoding sequence is contained in SEQ. ID NO:1.

42. The polynucleotide of claim 36, wherein the encoding sequence is contained in SEQ. ID NO:3.

43. A polynucleotide according to claim 36, wherein the polynucleotide encodes at least 5 consecutive amino acids of a complementarity determining region (CDR).

44. A polynucleotide according to claim 36, wherein the polynucleotide is in a cloning vector.

45. A polynucleotide according to claim 36, wherein the polynucleotide is an expression vector.

46. The expression vector of claim 45, wherein the expression vector is vaccinia.

47. A host cell comprising a polynucleotide according to claim 36.

48. A polypeptide having immunological activity of monoclonal antibody GM4-IgG4.λ, wherein the polypeptide comprises at least 5 consecutive amino acids from a variable region of monoclonal antibody GM4-IgG4.λ.

49. A polypeptide according to claim 48, wherein the variable region is from a light chain.

50. A polypeptide according to claim 48, wherein the variable region is from a heavy chain.

51. The polypeptide of claim 48, wherein the 5 consecutive amino acids is contained in SEQ. ID NO:2.

52. The polypeptide of claim 48, wherein the 5 consecutive amino acids is contained in SEQ. ID NO:4.

53. A polypeptide of claim 48, wherein the 5 consecutive amino acids are from a complementarity determining region (CDR).

54. A fusion polypeptide comprising the polypeptide of claim 48.

55. The fusion polypeptide of claim 54, comprising at least 10 consecutive amino acids of SEQ. ID NO:2 and at least 10 consecutive amino acids of SEQ. ID NO:4.

56. The fusion polypeptide of claim 55, wherein the amino acids of SEQ. ID NO:2 and the amino acids of SEQ. ID NO:4 are joined by a linker polypeptide of 5 to 20 amino acids.

57. The fusion polypeptide of claim 54, comprising a light chain variable region and a heavy chain variable region of monoclonal antibody H11.

58. The fusion polypeptide of claim 54, further comprising a cytokine.

59. The fusion polypeptide of claim 58, wherein the cytokine is GM-CSF.

60. The fusion polypeptide of claim 58, wherein the cytokine is IL-2.

61. The fusion polypeptide of claim 48 further comprising a heterologous immunoglobulin constant region.

62. A humanized antibody comprising the polypeptide of claim 48.

63. A polymeric GM4-IgG4.λ polypeptide comprising a plurality of the polypeptide of claim 48.

64. A pharmaceutical composition comprising monoclonal antibody GM4-IgG4.λ of claim 32 in a pharmaceutically acceptable excipient.

65. A pharmaceutical composition comprising the polynucleotide of claim 36 and a pharmaceutically acceptable excipient.

66. A pharmaceutical composition comprising the polypeptide of claim 48 and a pharmaceutically acceptable excipient.

67. An immunogenic composition comprising monoclonal antibody GM4-IgG4.λ of claim 32 and a pharmaceutically acceptable excipient.

68. A vaccine comprising the polynucleotide of claim 36 and a pharmaceutically acceptable excipient.

69. A vaccine comprising the polypeptide of claim 48 and a pharmaceutically acceptable excipient.

70. The vaccine of claim 67, further comprising an adjuvant.

71. The vaccine of claim 68, wherein the polynucleotide is comprised in a viral expression vector.

72. The vaccine of claim 71 wherein the viral expression vector is vaccinia.

73. A method of eliciting an immune response in an individual comprising administering to the individual an effective amount of the monoclonal antibody GM4-IgG4.λ of claim 32.

74. A method of eliciting an immune response in an individual, comprising administering to the individual an effective amount of the polynucleotide of claim 36.

75. A method of eliciting an immune response in an individual comprising administering to the individual an effective amount of the polypeptide of claim 48.

76. A method of treating cancer-associated disease in an individual comprising administering to the individual an effective amount of the monoclonal antibody GM4-IgG4.λ of claim 32.

77. A method of treating cancer-associated disease in an individual comprising administering to the individual an effective amount of the polynucleotide of claim 36.

78. A method of treating cancer-associated disease in an individual, comprising administering to the individual an effective amount of the polypeptide of claim 48.

79. The method of claim 76, wherein the cancer-associated disease is selected from the group consisting of melanoma, neuroblastoma, glioma, soft tissue sarcoma, and small cell carcinoma.

80. The method of claim 76, wherein the individual has a clinically detectable tumor.

81. The method of claim 76, which is a method for palliating the cancer-associated disease.

82. The method of claim 76, wherein a tumor that was previously detected in the individual has been treated and is clinically undetectable at the time of the administering of the monoclonal antibody GM4-IgG4.λ.

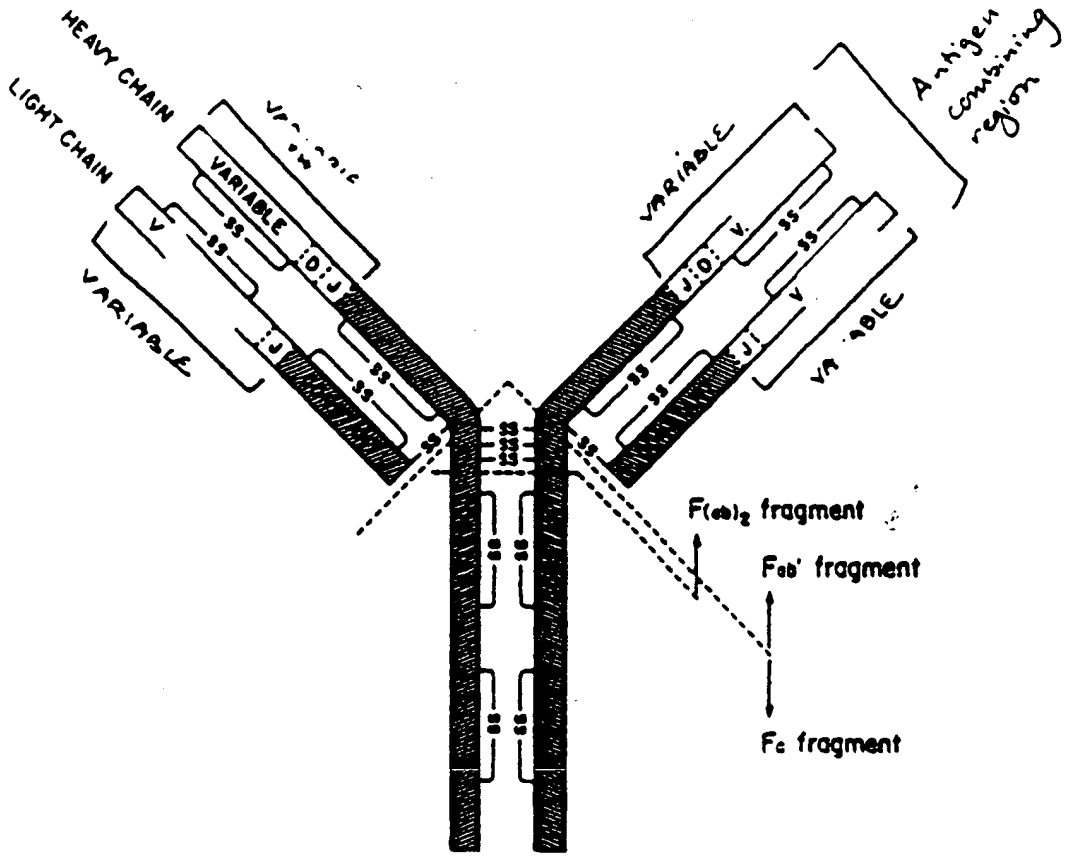
HEAVY CHAIN
LIGHT CHAIN
VARIABLE
VARIABLE
VARIABLE
VARIABLE
Antigen combining region
F(ab)2 fragment
Fab' fragment
Fc fragment

FIG. 1

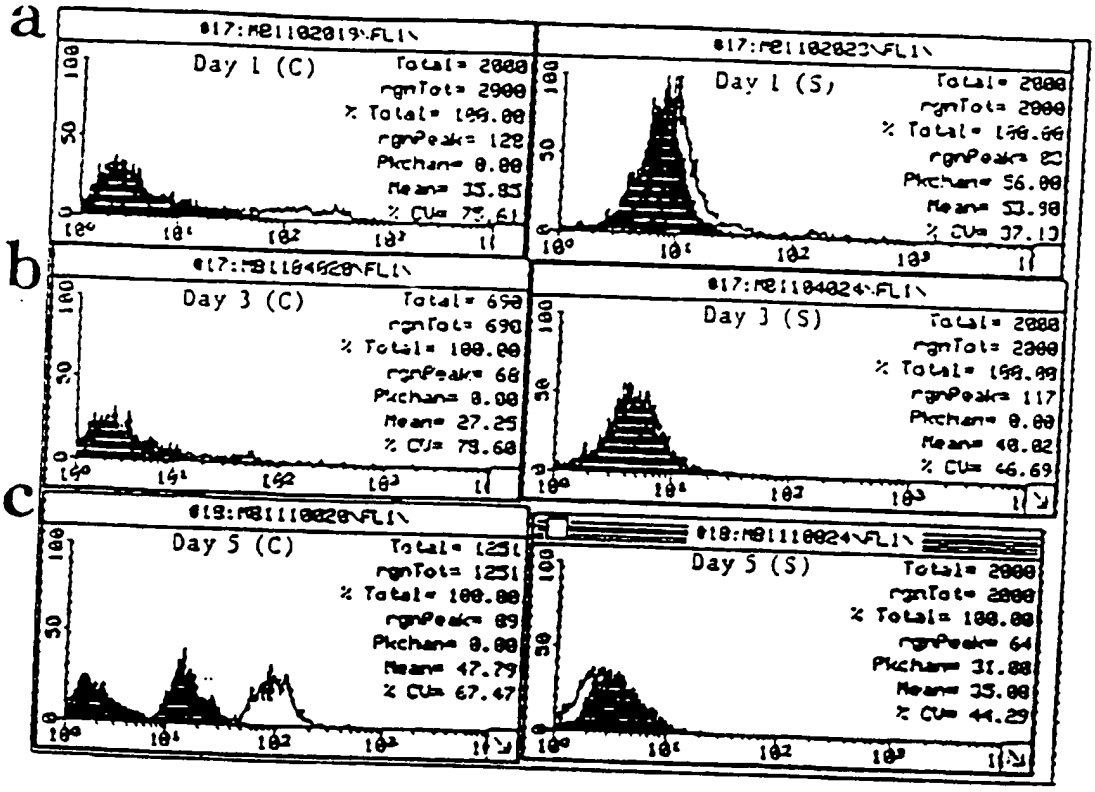

Figure 2. Flow cytommetry analysis of membrane and cytoplasmic AgGM4 expression on MENMEL94. a. membrane and cytoplasmic staining on Day 1; b. membrane and cytoplasmic staining on Day 3; c. membrane and cytoplasmic staining on Day 5. The shaded curve represents background fluorescence.

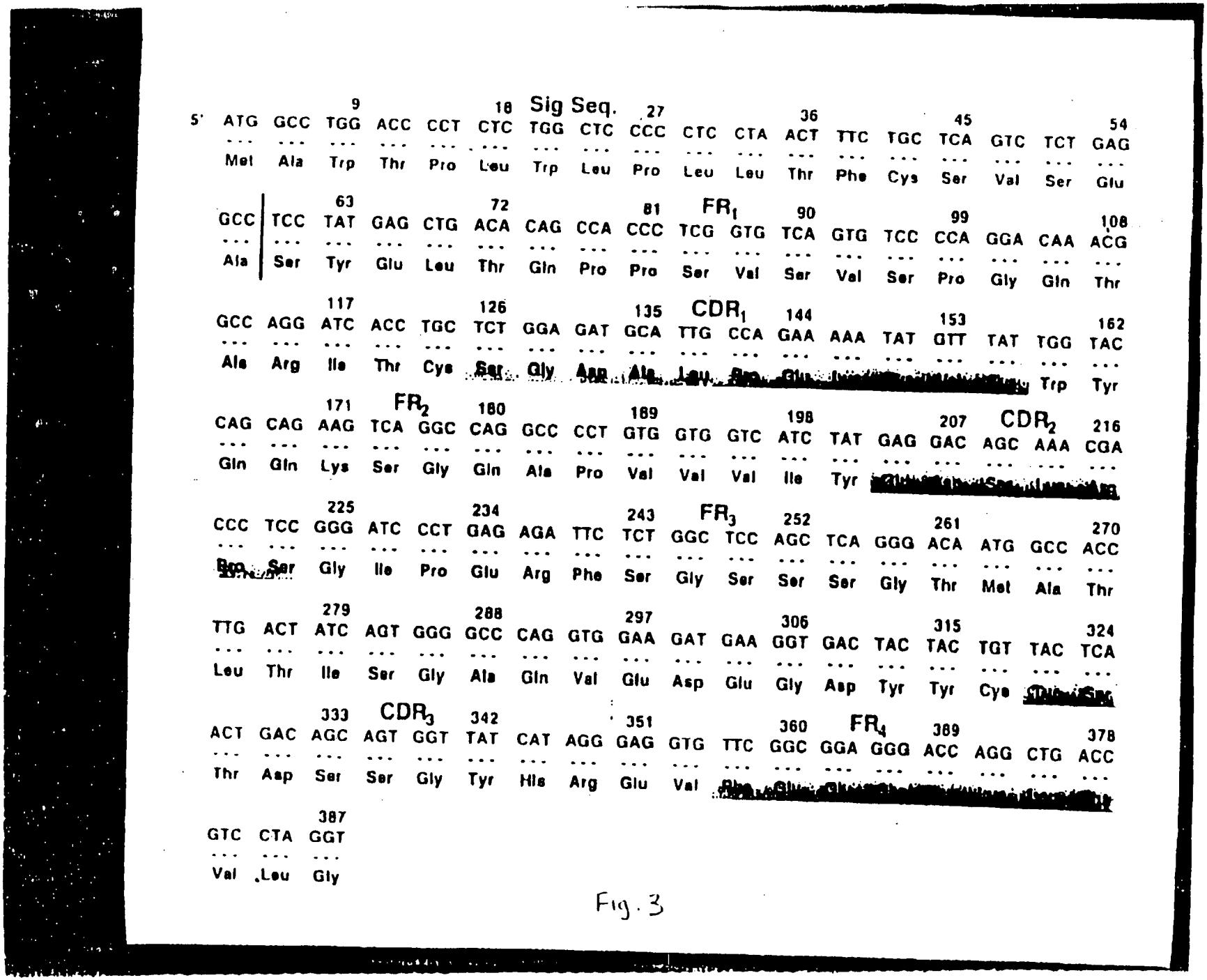

Fig. 3

Sig Seq.

5'	ATG	GAG	TTT	GGG	CTG	AGC	TGG	GTT	TTC	CTT	GCT	GCT	ATT	TTA	AAA	GGT	GTC	CAG
			9			18			27			36			45			54
	Met	Glu	Phe	Gly	Leu	Ser	Trp	Val	Phe	Leu	Ala	Ala	Ile	Leu	Lys	Gly	Val	Gln
	TGT	GAG	GTG	CAG	CTC	GAG	GAG	TCG	GGG	GGA	GGC	TTG	GTA	AAG	CCG	GGG	GGG	TCC
			63			72			81	FR_1		90			99			108
	Cys	Glu	Val	Gln	Leu	Glu	Glu	Ser	Gly	Gly	Gly	Leu	Val	Lys	Pro	Gly	Gly	Ser
	CTT	AGA	GTC	TCC	TGT	GCA	GCC	TCT	GGA	TTC	ACT	TTC	AGA	AAC	GCC	TGG	ATG	AGC
			117			126			135			144			153	CDR_1		162
	Leu	Arg	Val	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Arg	Asn	Ala	Trp	Met	Ser
	TGG	GTC	CGC	CAG	GCT	CCA	GGG	AAG	GGG	CTG	GAG	TGG	GTC	GGC	CGT	ATT	AAA	AGC
			171			180	FR_2		189			198			207			216
	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val	Gly	Arg	Ile	Lys	Ser
	AAA	ATT	GAT	GGT	GGG	ACA	ACA	GAC	TAT	CCT	GCA	CCC	GTG	AAA	GGC	AGA	TTC	ACC
			225			234	CDR_2		243			252			261			270
	Lys	Ile	Asp	Gly	Gly	Thr	Thr	Asp	Tyr	Pro	Ala	Pro	Val	Lys	Gly	Arg	Phe	Thr
	ATC	TCA	AGA	GAT	GAT	TCA	AAA	AAC	ACG	TTA	TAT	CTG	CAA	ATG	AAT	AGC	CTG	AAA
			279			288			297	FR_3		306			315			324
	Ile	Ser	Arg	Asp	Asp	Ser	Lys	Asn	Thr	Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Lys
	GCC	GAG	GAC	ACA	GCC	GTA	TAT	TAC	TGT	ACC	ACG	GGG	ATT	ATG	ATA	ACA	TTT	GGG
			333			342			351			360			369	CDR_3		378
	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	Thr	Thr	Gly	Ile	Met	Ile	Thr	Phe	Gly
	GGA	GTT	ATC	CCT	CCC	CCG	AAT	TGG	GGC	CAG	GGA	ACC	CTG	GTC	ACC	GTC	TCC	TCA
			387			396			405			414	FR_4		423			432
	Gly	Val	Ile	Pro	Pro	Pro	Asn	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser

Fig. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/06665

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN
search terms: rtlliktv

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HESS, J.F. et al. Nucleotide Sequence of the Bovine Vimentin-encoding cDNA. Gene. 25 March 1994, Vol. 140, No. 2, pages 257-259, see Figure 2.	29-31
X	HONORE. B. et al. Nucleotide Sequence of cDNA Covering the Complete Coding Part of the Human Vimentin Gene. Nucleic Acid Research. 1990, Vol. 18, No. 22, page 6692. see second paragraph and the figure.	29-31
Y	HARRIS, W.J. Therapeutic Antibodies-the Coming of Age. TIBTECH. February 1993, Vol. 11, pages 42-44, see page 43.	1

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
18 JULY 1997	29 AUG 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer SHEELA J. HUFF Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/06665

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 39/395, 39/40, 39/42, 38/00, 39/00; G01N 33/53, 33/542, 55/543; C07K 5/00, 7/00, 17/00, 16/00; C07H 21/04

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

530/324, 350, 387.2, 388.15, 388.1; 435/7.1, 7.9, 326, 331, 320.1; 424/130.1, 131.1, 133.1, 134.1, 141.1, 142.1; 536/23.53; 436/518, 64

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

530/324, 350, 387.2, 388.15, 388.1; 435/7.1, 7.9, 326, 331, 320.1; 424/130.1, 131.1, 133.1, 134.1, 141.1, 142.1; 536/23.53; 436/518, 64